Prolactin: Is It an Essential Hormone for Maternal Behavior in the Mammal?¹

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Evidence is reviewed for the concept that prolactin is one of the essential hormones for maternal behavior in the rabbit, rat and mouse. Ergocornine hydrogen maleate prevents maternal nest building in the pregnant rabbit, but if prolactin is added to the treatment regimen, nest building occurs. In the rat, retrieval, crouching and licking of pups is obtained following treatment with estradiol, progesterone and prolactin. In the mouse, the evidence is not as strong but one laboratory has obtained retrieval following implantation of prolactin in the hypothalamus.

The basic work of Riddle and his colleagues (1935, 1942) suggested that prolactin is the hormone responsible for maternal behavior in the rodent. This concept has been challenged in recent years, but is still presented in numerous textbooks. Indeed, Beach and Wilson (1963) point out that “... in 1942 Riddle was not entirely convinced that prolactin was the primary hormone factor although he felt that it was the most likely effective agent.” Beach and Wilson go on to state, “This commendable restraint was not observed when the data were reviewed by other authors, or when they eventually were incorporated into textbooks.”

The role of prolactin in parental behavior in birds, including broodiness, incubation of eggs, and feeding of young, was first described by Riddle et al. in 1935. The stimulation of these behaviors by prolactin has been found in the domestic hen (Riddle et al., 1935), the pheasant (Crispens, 1956), the

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²Part of the data reported here were presented at the Prolactin Workshop held on January 1, 1971, Bethesda, MD.
turkey (Crispens, 1957), and the ring dove (Lehrman, 1958). For recent reviews see Eisner (1960) and Lehrman (1961). Studies on mammals have not been as extensive as those in birds. Attempts to confirm the findings of Riddle et al. in the rat (1935, 1942) have been negative. Beach and Wilson (1963) and Lott and Fuchs (1962) failed to initiate retrieval in virgin female rats by the injection of prolactin.

The present report will review data concerning a role for prolactin in maternal behavior in three species: the rat, mouse, and rabbit, and present new findings from our laboratory that point to prolactin as one of the essential hormones for certain aspects of maternal behavior in several species of mammals.

THE RAT

The involvement of prolactin in the initiation of retrieval of young in the rat had not been fully recognized until the past year or so. Although some positive evidence had been reported, most of the subsequent data failed to confirm the original findings and, indeed, have suggested that any hormonal involvement is questionable. Recently, Terkel and Rosenblatt (1969) induced maternal retrieving in virgin females by injecting them with 3-4 ml of plasma taken from lactating rats within 48 hr of parturition. The retrieval test was carried out by placing 5-10-day-old pups in the front of each cage. The rats were observed continually for 15 min and thereafter for 1 min at 20-min intervals up to 2 hr. This was repeated daily until retrieval occurred. Normally, a virgin rat shows pup-retrieval and other maternal behaviors after exposure to pups for approximately 7 days. The maternal plasma reduced the onset of retrieval by approximately 50% whereas plasma from rats in proestrus had no effect and plasma from diestrus rats actually increased the latency to retrieve. In addition, the animals showing retrieval also showed other components of maternal behavior including crouching over the pups, licking the young, and nest building.

In the past year Terkel (1970) has obtained immediate retrieval in virgin rats by an elegant procedure which employs a continuous blood exchange between two unrestrained rats. In this system one animal was pregnant while the other was a virgin. The virgin rat exhibited all the maternal behaviors as soon as its counterpart delivered its offspring.

It is obvious that plasma from a lactating mother (taken within 48 hr after parturition) contains one or more substances capable of inducing maternal behavior. Thus, these results point again to the possibility of hormonal involvement. However, until the active element or elements in the plasma are identified, it is impossible to conclude whether the endocrine system is involved, let alone prolactin.
In our initial studies we resurrected the classical approach of replacement therapy to induce maternal behavior. Employing the castrated virgin rat, we attempted to establish a hormonal balance typical of pregnancy and consisting of various ratios of estradiol and progesterone. The results were negative (Denenberg, Taylor, and Zarrow, 1969).

The current study involved the treatment of rats with a hormonal regimen designed to produce growth of the mammary gland and lactation. Castrated virgin rats were tested for retrieval and those which gave a positive response were eliminated. The remainder received sc injections of 2 μg estradiol and 4 mg progesterone daily for 20 days, followed by 2 mg cortisol acetate and 20 IU of prolactin daily until the termination of testing. All rats were tested for retrieval at 24 hr after the fourth injection of cortisol acetate and prolactin. At each day’s test, a female was exposed to three rat pups 2-8-days old and observed for pup retrieval, crouching, licking, etc. for a 15-min period. She was then left with the pups overnight. The following morning, the position of the pups in relation to the adult rat was noted. The pups were then replaced by fresh pups and the test carried out as before. Testing was continued until an animal retrieved pups for 2 consecutive days or until the 7th day.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Latency to retrieve days ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no RX)</td>
<td>14</td>
<td>4.1 ± 0.66</td>
</tr>
<tr>
<td>Control (oil + saline)</td>
<td>17</td>
<td>4.9 ± 0.55</td>
</tr>
<tr>
<td>E, P, Pro1 and C</td>
<td>17</td>
<td>1.4 ± 0.61</td>
</tr>
<tr>
<td>E, P, and C</td>
<td>10</td>
<td>1.8 ± 2.08</td>
</tr>
<tr>
<td>E, P, and Prol</td>
<td>10</td>
<td>1.4 ± 0.96</td>
</tr>
</tbody>
</table>

aE—estradiol; P—progesterone; C—cortisol acetate; Prol—prolactin.

The data obtained are presented in Table 1. The nontreated control rats and the control rats receiving oil and saline had a latency to retrieve of 4.1 and 4.9 days, respectively. Treatment with the full hormonal regimen significantly decreased the latency time to 1.4 days. Two of the 17 rats in this group failed to retrieve during the 7-day test period and were given an

4The prolactin (NIH-P-S-9 ovine) was obtained through the courtesy of NIAMD, NIH.
arbitrary score of 8 days as was done in all groups when some of the rats failed to retrieve. If we drop out the two failures, the average latency for the rats that retrieved drops to 0.6 days. If the data are plotted for the accumulated percentage of rats retrieving on each of the test days, it is apparent that all of the hormone-treated rats that are going to retrieve have done so by the second testing period (Fig. 1). However, an increase in the number of control rats that retrieved occurred throughout the entire 8-day test period.

The results indicate that a hormonal regimen capable of inducing full mammary gland growth also induces pup retrieval and related behavior such as anogenital licking and the assumption of a nursing posture. However, actual suckling by the pups was not observed. It is highly significant that 41% of the rats retrieved within the first 15 min on initial exposure to pups. This is approximately the same time seen in the animal following delivery. No reason can be given at this time for the failure of the others to retrieve immediately, but conceivably the optimum hormone ratios may not have been present.

The following experiment was designed to establish whether exogenous cortisol or prolactin was essential for the initiation of maternal behavior. Ovariectomized, adult, virgin rats were treated either with estradiol, progesterone, and prolactin; or estradiol, progesterone, and cortisol acetate. The dosages and the testing procedure were identical to those described previously.

Fig. 1. Percentage of virgin, castrate rats retrieving per day following treatment with vehicle (n = 14) or estradiol, progesterone, prolactin and cortisol (n = 14).
As seen in Table 1, animals treated with estradiol, progesterone and prolactin had a mean retrieval latency of 1.4 days, while the group treated with estradiol, progesterone and cortisol acetate had a mean latency of 1.8 days.

It is apparent that the addition of cortisol acetate to the treatment schedule had no significant effect. Since the animals possessed intact adrenal glands, we cannot rule out the presence of endogenous corticoids. We can only conclude that exogenous corticoids are not essential when the animal possesses an intact pituitary-adrenal system. Similarly, the decreased retrieval time obtained with the steroids with or without prolactin may be explained by the presence of endogenous prolactin and the ability of the sex steroids to cause increased plasma prolactin levels (Amenomori, Chen, and Meites, 1970). Ultimately, this experiment will have to be repeated with an hypophysectomized rat to establish beyond question the endocrine requirements for maternal behavior.

In an independent study performed at the same time we were doing our work, Moltz, Lubin, Leon, and Numan (1970) reported the induction of retrieval in the castrate virgin rat following treatment with 12 µg estradiol benzoate from Day 1 through Day 11, 3 mg progesterone twice daily on Days 6-9, and 50 IU of prolactin on the evening of Day 9 and the morning of Day 10. The animals were tested with 6 foster young on the afternoon of Day 10; this was repeated with a new litter on Day 11 and thereafter. The latency for the exhibition of maternal behavior by the fully treated rats was reduced from approximately 140 hr for the control rats to 40 hr for the hormone-treated rats. Treatment with the two steroids and no prolactin required approximately 72 hr before retrieval was observed.

In another experiment, we treated 14 pregnant rats with 1 mg/kg of ergocornine hydrogen maleate. The ergot derivative was used because of its ability to inhibit prolactin release and thereby to decrease the level of plasma prolactin (Shelesnyak, 1955, 1958). Treatment was begun on Day 17 of gestation and was continued through parturition and until the death of the pups which occurred within 72 hr of delivery. In every case, the ergocornine-treated subjects retrieved pups and assumed nursing postures over them, even though milk was not available for lactation. Examination revealed a lack of milk in the mammary glands of the rat mothers and at no time was milk found in the stomachs of the pups. Hence, it may be safely assumed the rat pups died of starvation. In view of the ability of ergocornine derivatives to inhibit prolactin release and the present results indicating a lack of lactation, it might be assumed that prolactin is not involved in maternal behaviors. However, while plasma levels of prolactin are depressed following ergocornine, measurable levels of the hormone are still present in the blood. Hence,
adequate prolactin may be available for the presentation of maternal behaviors but not enough for lactation. A final answer will require the use of the hypophysectomized rat, but in view of the finding that treatment with the steroid hormone induces maternal behavior and that this treatment causes prolactin release, it may be inferred that prolactin is an essential hormone for maternal behavior in the rat.

**THE MOUSE**

One aspect of maternal behavior namely, brood-nest building, has been shown to be induced by progesterone (Koller, 1952, 1956; Lisk et al., 1969). However, no one had suggested a possible need for prolactin. Voci and Carlson (1971) have suggested that prolactin is involved in the exhibition of maternal behavior in the mouse. They implanted 70 μg of prolactin into the hypothalamus, the neocortex, or under the skin. In other subjects, 70 μg of progesterone were implanted into the hypothalamus. Mice implanted with prolactin in the hypothalamus or subdermally showed a significant increase in maternal behaviors as measured by speed of retrieval, time in nest, licking of the pups, and nest quality. A group of mice treated with progesterone implanted into the hypothalamus built superior nests, but their retrieval performance was indistinguishable from that of the controls. These results provide the only evidence available to date for the role of prolactin in inducing maternal behavior in the mouse.

The finding that progesterone would induce superior nest building without affecting retrieval is most interesting. Such a separation indicates that the two events are induced through separate mechanisms which are synchronized in time. Although confirmation of the finding of Voci and Carlson is necessary before final conclusions can be drawn, the results indicate that prolactin may also be essential for maternal behavior in the mouse.

**THE RABBIT**

A highly characteristic pattern of maternal behavior in the rabbit (Oryctolagus cuniculus, L.) is the building of a nest formed of straw, hay, or comparable materials and lined with hair plucked from a mother’s body. The young are deposited in this nest and usually covered with hair. In the normal course of events, the animal initially builds a straw nest at the beginning of the third trimester of gestation. Hair loosening occurs just prior to parturition and plucking body hair and lining of the straw nest occurs in our strains (Dutch-belted and Florida White) at the time of delivery. The hair-lined nest is referred to as a maternal nest and is present not only at the end of gestation,
but also to a limited extent at the termination of pseudopregnancy. Neither hair loosening nor formation of a maternal nest occur in the male.

The degree of hair loosening can be determined by making three passes with a steel comb along each side of the body and flank of the animal and weighing the amount of hair obtained (Sawin et al., 1960). Significant hair loosening was found to occur both in the pregnant and pseudopregnant animal. In the pregnant animal this can be observed 90% of the time which correlates well with the frequency of maternal nest building. Treatment with estradiol, progesterone, and prolactin resulted in a significant peak in hair loosening (Faroq et al., 1963). However, a treatment period of 56 days was used and no effort made to reduce the length of treatment to physiological limits.

Maternal nest building in the rabbit has also been studied in great detail and the hormonal requirements established (Zarrow et al., 1961, 1963). Table 2 summarizes some of the data. In all instances estradiol treatment was started on Day 1 and progesterone on Day 2. Progesterone treatment was terminated at the times given and the estrogen continued. Maternal nest building usually occurred from 24 to 72 hr after the cessation of progesterone treatment. These tests were carried out in ovariectomized rabbits of the Dutch-belted and Grey Chinchilla strains. It is apparent that both strains of rabbits build maternal nests following treatment with estradiol and progesterone and that the larger strain (Grey Chinchilla) needed a longer treatment period. Nevertheless, in both instances the treatment period was less than the 32-day gestation period found in the rabbit. It should also be noted that the treatment was designed to replicate the hormonal changes seen in steroid levels during pregnancy and that maternal nest building occurred when the hormonal regimen had shifted from one of progesterone dominance to one of estrogen dominance.

### TABLE 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Length of treatment</th>
<th>% that built nests</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>E-μg</td>
<td>P-mg</td>
<td>N</td>
</tr>
<tr>
<td>Dutch-belted</td>
<td>5</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Grey Chinchilla</td>
<td>5</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4</td>
<td>29</td>
</tr>
</tbody>
</table>

\* \*Taken from Zarrow et al., 1965. \* \*
The above experiments were carried out in ovariectomized rabbits so that endogenous ovarian contributions to the hormonal state of the animal could be ruled out. However, the rabbits were not hypophysectomized so that it could not be stated whether the pituitary gland secreted a hormone or hormones essential for maternal behavior. In a second set of experiments, the rabbits were ovariectomized first and treated with estradiol and progesterone to demonstrate the ability of each test animal to build a maternal nest. The animals were then hypophysectomized via the parapharyngeal approach of Rennie et al., (1964) and allowed several weeks for recovery and were then given a second course of hormonal treatment. The Dutch-belted rabbits were treated with 5 µg estradiol for 20 days and 2 mg of progesterone on Days 2-15 of estrogen treatment. The hybrid rabbits (Dutch-belted X New Zealand White) received 10 µg estradiol daily for 20 consecutive days plus 4 mg progesterone on Days 2-15. When the animal was given more than one test, a minimum of 5 days intervened between the last estradiol injection and the beginning of the next test. All females were provided with a nest box (19 X 21.5 X 10 in.) and ad lib. hay nest construction 13 days postoperatively and 1 day prior to treatment.

The results showed that none of the ovariectomized-hypophysectomized rabbits of either the Dutch-belted group or the hybrid group built maternal nests following treatment with estradiol and progesterone (Table 3). Both of the hybrid ovariectomized-sham-hypophysectomized Ss built nests following the first treatment period; whereas, the number of Purdue-Dutch, ovariectomized, sham-hypophysectomized rabbits that built nests increased from 1 out of 6 (17%) at the first treatment period to 4 out of 6 (67%) at the second period and to 5 out of 6 (83%) at the third treatment period, a highly significant increase (Anderson et al., 1971). If the data for the groups are

<table>
<thead>
<tr>
<th>Treatment period</th>
<th>New Zealand X Dutch-belted</th>
<th>Dutch-belted</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Ratio of number of rabbits that built maternal nests over the number tested</td>
<td>AP&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/6</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0/6</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0/6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Taken from Anderson et al., 1971.
<sup>b</sup>Treatment period refers to the three different tests for steroid-induced maternal behavior given the Dutch-belted rabbits.
<sup>c</sup>Hypophysectomized.
averaged, we find that none of 12 ovariectomized-hypophysectomized rabbits built maternal nests; whereas 7 out of 8 (87%) of the ovariectomized sham-hypophysectomized rabbits eventually built nests. This difference indicates that the presence of the pituitary gland is essential for maternal nest building.

In a current set of experiments, maternal nest building was examined in rabbits treated with ergocornine hydrogen maleate. Rabbits of the Florida White strain were used and all animals were checked for nest building prior to and after the course of the treatment. Treatment with the ergot derivative, im, once daily was started on the 26th day of gestation and two doses were used (0.5 and 1.0 mg/kg body wt). The ergocornine hydrogen maleate was suspended in sesame oil and injected im. Two out of 6 rabbits treated with 0.5 mg ergocornine hydrogen maleate/kg body wt built maternal nests and none of 6 rabbits built maternal nests following treatment with 1 mg ergocornine hydrogen maleate/kg body wt (Table 4). Delivery appeared to be normal and gestation length was not significantly affected. At the lower dose of the ergocornine, all the pups from the 4 litters where the mothers failed to build maternal nests died within 3 days after delivery. The litters from the two does that built maternal nests, albeit poor ones, remained alive to weaning. The average body weight curves for these pups was markedly inferior to that of the pups from the control mothers (Fig. 2), which could be due to an inadequate release of prolactin for maintenance of normal lactation. At the higher dose of ergocornine, the majority of the pups were born alive but all were dead within 3 days. Little to no milk could be seen in the stomachs of the pups.

In a second experiment, pregnant rabbits were treated with 1 mg ergocornine hydrogen maleate/kg body wt starting on Day 26 of gestation as above and in addition with 4 mg prolactin twice daily starting on Day 27 of gestation. Four of the 5 rabbits (80%) treated with prolactin and ergocornine built maternal nests; whereas none of 6 (0%) built maternal nests when treated with 1 mg ergocornine alone. Obviously prolactin prevented the

<table>
<thead>
<tr>
<th>No. of rabbits</th>
<th>Ergocornine HM mg/kg</th>
<th>Prolactin</th>
<th>Gestation days</th>
<th>% rabbits with maternal nest</th>
<th>Pups born Alive</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>31.1</td>
<td>100</td>
<td>51</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>-</td>
<td>31.5</td>
<td>33</td>
<td>28</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>-</td>
<td>30.3</td>
<td>0</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>4 mg</td>
<td>29.5</td>
<td>80</td>
<td>7</td>
<td>21</td>
</tr>
</tbody>
</table>

*Twice daily starting on Day 27.*
inhibitory effect of ergocornine on maternal nest building in the pregnant rabbit and would appear to be the hormone from the pituitary gland that is essential for maternal nest building. It is of interest to note that gestation length was reduced following treatment with ergocornine and prolactin and that a greater number of pups was born dead. Additional work is underway to examine this finding in greater detail.

It is apparent that the treatment with ergocornine derivative inhibited nest building as well as lactation. A possible explanation for this action of the drug is the inhibition of prolactin release. This would imply that prolactin is an essential factor for maternal behavior. However it must be kept in mind that the drug has multiple effects and the inhibition of maternal behavior could be due to an effect other than interference with prolactin release. Nonetheless, the available data point to a role of prolactin in the expression of maternal behavior in the rabbit.
CONCLUSIONS

The original proposal by Riddle that prolactin is an essential factor for the presentation of maternal behavior in the mammal may be valid. Although experimenters for the past 15 years have been unable to repeat the findings of Riddle, current data from our laboratory and the laboratories of others indicate that prolactin plays an essential role in certain aspects of maternal behavior in the rabbit. The data in the rat and mouse are not as strong and direct as those in the rabbit but by inference prolactin appears to be involved. While a full understanding of the hormonal involvement in maternal behavior in the mammal is still to be sought, we can now state that both the female sex steroids and prolactin appear to be involved in the expression of maternal behavior.

REFERENCES


